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HPV 16 ANTIBODY PREVALENCE IN JAMAICA AND THE UNITED STATES REFLECTS DIFFERENCES IN CERVICAL CANCER RATES

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Human papillomavirus (HPV) is widely accepted as the primary etiologic agent in the development of cervical cancer. DNA of a particular HPV type, HPV 16, is found in about half of tumors tested. Inconsistent with this causal relationship, however, population-based studies of HPV DNA prevalence have often failed to find high rates of anogenital HPV infection in countries with high cervical cancer rates. To examine this issue, we used serology to compare HPV 16 exposure in healthy volunteer blood donors in the United States (n = 278) and similar subjects from a country with 3-fold higher cervical cancer rates, Jamaica (n = 257). Jamaican sexually transmitted disease (STD) patients (n = 831) were also studied to examine in detail the relation of HPV 16 antibodies with sexual history. Serology was conducted using an ELISA employing HPV 16 virus-like particles (VLPs). Age-adjusted seroprevalence rates were greatest among male (29%) and female (42%) STD patients, intermediate in male (19%) and female (24%) Jamaican blood donors and lowest among male (3%) and female (12%) U.S. blood donors. The higher seroprevalence in women was significant, and prevalence tended to increase with age. In multivariate logistic regression, controlling for age and gender, Jamaican blood donors were 4.2-fold (95% CI 2.4–7.2) and STD patients 8.1-fold (95% CI 5.0–13.2) more likely to have HPV 16 VLP antibodies than U.S. blood donors. Among STD patients, HPV 16 antibodies were associated with lifetime number of sex partners and years of sexual activity, as well as other factors. Our data suggest that HPV 16 VLP antibodies are strongly associated with sexual behavior. Moreover, exposure to HPV 16 appears to be much greater in Jamaica than in the United States, consistent with the high rate of cervical cancer in Jamaica. *Int. J. Cancer* 80:339–344, 1999.
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Infection with human papillomavirus (HPV) is considered a central causal factor in cervical cancer tumorigenesis (Muñoz *et al.*, 1992; Schiffman *et al.*, 1993; Bosch *et al.*, 1995). There are over 70 HPV types, and more than 20 of these commonly infect anogenital epithelial tissues through sexual transmission. A single HPV type, HPV 16, is believed to be involved in about half of all cervical cancer cases (Bosch *et al.*, 1995). HPV 16 is also the most frequent type of cervical HPV infection in normal, healthy women.

HPV infection is generally detected using sensitive DNA-hybridization methods, such as PCR. Over 90% of cervical cancer specimens have been shown to contain HPV DNA using these assays (Schiffman *et al.*, 1993; Bosch *et al.*, 1995). However, DNA testing has important limitations. Most HPV infections are transient and the DNA becomes undetectable (Schneider and Koutsky, 1992), making DNA hybridization a poor measure of cumulative lifetime exposure to HPV. Possibly because of this, several ecological population-based studies have not demonstrated greater HPV DNA prevalence (reflecting greater virus exposure) in populations with high incidence of cervical cancer (Kjaer and Jensen, 1992; Svare *et al.*, 1998). These findings, nevertheless, are inconsistent with the central role of HPV in neoplasia of the cervix, and it remains to be proven that HPV exposure is greater in populations with high cervical cancer rates.

Antibodies to HPV infection are likely to persist for an extended (if undefined) period, making serology a potentially better measure of cumulative HPV exposure (Carter *et al.*, 1996). ELISAs based on HPV 16 virus-like particles (VLPs) have been shown to detect antibodies strongly associated with anogenital HPV 16 infection (Kirnbauer *et al.*, 1994; Carter *et al.*, 1996), and laboratory evidence supports the premise that these antibodies are type-specific (Wang *et al.*, 1997). Seropositivity is greatest in women with persistent HPV 16 infections (Carter *et al.*, 1996) and in those who progress to high-grade cervical neoplasia (de Gruijl *et al.*, 1997; Svare *et al.*, 1997), both important risk factors in the development of cervical cancer. However, few serologic studies have been conducted to confirm that HPV 16 exposure, as measured by HPV 16 VLP seroprevalence, is (as expected) greater in populations with high cervical cancer incidence rates (Nonnenmacher *et al.*, 1995) and few studies have tested the usefulness of HPV serology as a marker of sexual HPV exposure in men (Svare *et al.*, 1997).

Therefore, in this investigation, we measured HPV 16 VLP antibody prevalence in two populations with contrasting cervical cancer incidence rates, testing subjects from the United States (incidence 7.8 per 100,000 person-years) and from Jamaica, a Caribbean nation with 3-fold higher cervical cancer rates (incidence 26.8 per 100,000 person-years) (Brooks *et al.*, 1995; Reis *et al.*, 1997). Specifically, in both countries, we studied healthy volunteer blood donors, a convenience sample that is commonly used to estimate seroprevalence in the general population as part of international sentinel serosurveys (Strickler *et al.*, 1995). Furthermore, to demonstrate the strong sexual association of the antibody responses being measured, we contrasted the results in blood donors to seroprevalence rates in Jamaican sexually transmitted disease (STD) patients and, in these patients, examined risk factors for positive antibody responses.

MATERIAL AND METHODS

Subjects

STD patients. Subjects were consecutive patients with new complaints during 1994 and 1995, presenting to the Comprehensive Health Center, the main clinic offering specialized services for STDs in Kingston, Jamaica. Patients attending this clinic are almost exclusively black and of middle to lower socio-economic status. Patient recruitment and evaluation procedures have been previously described (Figueroa *et al.*, 1995). In brief, each subject provided serum and was administered a detailed questionnaire to obtain demographic data and information about sexual history. The

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questionnaire was slightly different for men and women. From the 1,455 initially enrolled subjects, we used stratified random sampling to achieve a gender-balanced study population. There were 404 male and 427 female STD patients in our final analyses (see ''Statistical analysis'', for discussion of subjects with indeterminate results).

Jamaican blood donors. The subjects studied were 141 male and 116 female consecutive blood donors enrolled through Kingston blood banks between 1987 and 1988. Over 95% of subjects identified their ethnic group as black. The sera were collected as part of a study to measure the prevalence of human T-cell lymphotropic virus type-I (HTLV-I) infection in blood donors. HTLV-I-seropositive subjects were excluded from the current analysis to preserve these limited specimens, but this involved few individuals (2.4% of all donors) (Manns *et al.*, 1992).

US blood donors. Sequential healthy volunteer blood donors were enrolled during 1996 and 1997 through the National Institutes of Health, Bethesda, Maryland as part of a program to collect blood specimens for research purposes. A random subset of these subjects were selected to obtain comparable numbers of males (n = 140) and females (n = 138) for analysis in the current study.

Laboratory methods

HIV and HTLV-I serology. All serology for retroviral infections was conducted using commercially available assays, according to manufacturers' instructions, with initial screening by ELISA and confirmation of positive antibody reactivity by Western blot.

HPV-16 VLP ELISA. HPV-16 VLPs were prepared from Sf9 insect cells infected with a recombinant baculovirus expressing the L1 and L2 proteins of HPV-16 and purified by the method of Kirnbauer *et al.* (1994). The VLPs were then diluted to 2 µg/ml in PBS (GIBCO, Grand Island, NY), and the VLP solution was added to each of 96 wells in a polystyrene PolySorp plate (Nunc, Naperville, IL) in 100-µl volumes. Following overnight incubation at 4°C, plates were washed 3 times using wash solution [0.05% Tween 20 and 0.01% Triton X-100 (Sigma, St. Louis, MO) in PBS] in an automatic plate washer (Microwash 2; Skatron, Lier, Norway). Plates were tapped dry on paper towels and 300 µl per well of blocking solution (5% BSA in PBS) added. Following incubation at 37°C for at least 3 hr, plates were washed 3 times as described. Serum samples were diluted 1:20 in blocking solution and added in 100-µl volumes to each well. Plates were covered with a plate sealer, incubated for 1 hr at 37°C and then washed 5 times as described above. Goat anti-human IgG conjugated with alkaline phosphatase (Boehringer-Mannheim, Indianapolis, IN) was diluted 1:3,500 in blocking solution and 100 µl were added to each well. Plates were covered with a plate sealer, incubated for 30 min at 37°C and washed 5 times. Substrate solution [1 mg/ml 1-para-nitrophenylphosphate in diethanolamine buffer, pH 9.8 (Sigma)] was added to each well in 100-µl volumes. Plates were covered and incubated for 30 min at 37°C. The enzyme reaction was stopped by the addition of 50 µl of 3N NaOH per well. Plates were then read at 405 nm in an automated ELISA plate reader (model EL312e; Bio-Tek, Winooski, VT) to determine optical density (OD) values.

To prevent batch effects, the duplicate serum samples tested in this study were placed in mixed order on the ELISA plates. Laboratory personnel were masked to all patient information. To control for intra-laboratory day-to-day variability, sample OD values were adjusted according to OD results in 3 control specimens tested in triplicate on each individual plate: one high, one intermediate and one low responder. Positive results in study specimens were determined by applying cut-points set in previous investigations (positive OD $>$ 1.017, negative OD $<$ 0.904) to the mean-adjusted OD value for each duplicate sample (Strickler *et al.*, 1997). Furthermore, to minimize misclassification, borderline results (*i.e.*, 0.904–1.017) were *a priori* classified as indeterminate (7% of all subjects tested), as suggested by our earlier results.

Statistical analysis

Exploratory data analysis showed that indeterminate (borderline) results in the HPV 16 VLP ELISA were more common in Jamaican STD patients (10%) than in Jamaican blood donors (5%) and US blood donors (3%), suggesting that at least some of these ''indeterminate'' results were probably true positives. *A priori* exclusion of individuals with indeterminate findings, therefore, likely under-estimates the prevalence of HPV antibodies in the STD patients and the magnitude of the differences between the study groups (bias toward the null). However, in previous studies of populations with low seroprevalence, we have shown that this approach may reduce misclassification and improve inter-laboratory agreement, making results more generalizable (Strickler *et al.*, 1997). In any case, our preliminary analyses in this study showed that the overall findings were not meaningfully altered by redefining indeterminate serologic results as negative, as positive, as an intermediate category, or by excluding them (our set approach for dealing with these uncertain observations). Therefore, the seroprevalence data are presented as the fraction of subjects with interpretable (non-indeterminate) results.

Direct adjustment was used to control for age effects, applying the age distribution of all subjects combined to determine the relative weights of each age strata. Statistical significance in contingency tables was determined using Pearson's χ^2 or Fisher's exact test, as appropriate based on the expected values. Linear trends were assessed using the Mantel extension test, and multivariate analyses were conducted using logistic regression methods. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated to further measure the strength of associations. Differences between groups in continuous variables were determined by Student's *t*-test and by multivariate ANOVA, whereas correlations were measured using Pearson's coefficient. All data analysis was performed using standard commercial statistical software.

RESULTS

Comparisons of blood donors and STD patients

Table I summarizes the age and gender characteristics of all subjects studied. The mean age of blood donors in the United States was greater than that in Jamaica ($p < 0.001$, in a multivariate ANOVA adjusting for gender), and Jamaican STD patients had the lowest mean age ($p < 0.05$). Males were significantly older than females within each study group (all p values < 0.01).

To compare HPV 16 VLP seroprevalence in these populations, we calculated their age- and gender-specific antibody-prevalence rates. These data are shown in Figure 1. Age-adjusted seroprevalence rates were greatest among male (29%) and female (42%) STD patients, intermediate in male (19%) and female (24%) Jamaican blood donors and lowest among male (3%) and female (12%) US blood donors. Notably, these differences were consistently observed at all ages in both sexes, although the magnitude of the differences varied. In multivariate logistic regression, Jamaican blood donors were 4.2-fold (95% CI 2.4–7.2) and STD patients 8.1-fold (95% CI 5.0–13.2) more likely to have HPV 16 VLP antibodies than US blood donors, after adjusting for age and gender.

In general, within each study group, HPV 16 seroprevalence rates increased with age (Fig. 1). Specifically, among STD patients, the relation with age was highly significant in both men ($p < 0.01$) and women ($p < 0.001$). Among blood donors, the age-specific seroprevalence pattern was more variable, and only in male blood donors from Jamaica ($p = 0.01$) was this relationship statistically significant. Furthermore, in blood donors, the age-specific trends suggested that these increases might plateau. However, additional

TABLE I – AGE AND GENDER CHARACTERISTICS OF THE STUDY POPULATIONS

	Males		Females	
	N	Mean age (range)	N	Mean age (range)
US blood donors	140	40.1 (17–72)	138	34.4 (17–78)
Jamaican blood donors	141	30.4 (17–80)	116	27.3 (17–59)
Jamaican STD patients	404	28.3 (15–70)	427	26.4 (14–61)

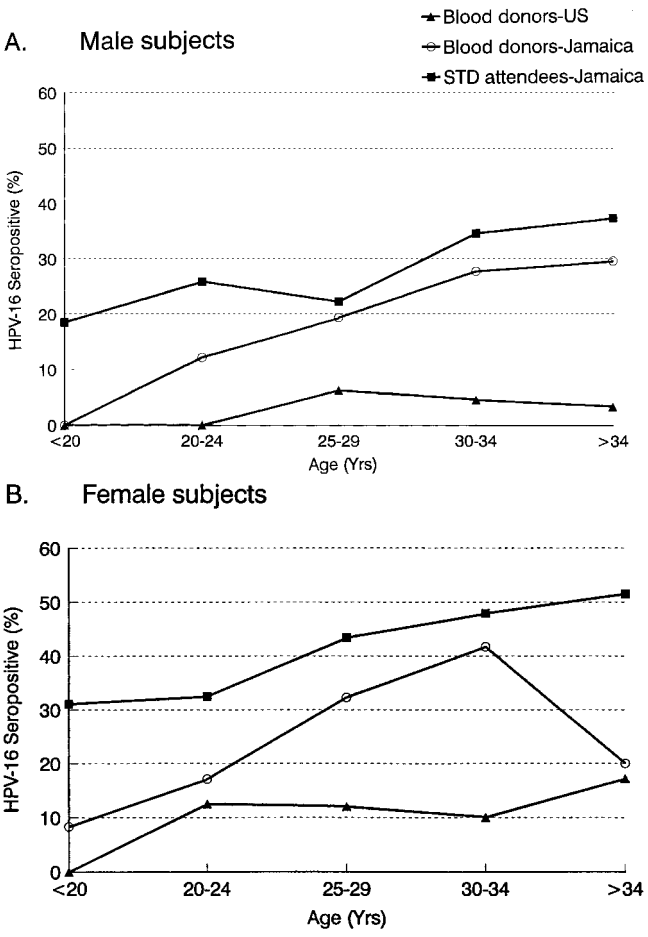

FIGURE 1 – Age-specific HPV 16 seroprevalence rates in *(a)* male and *(b)* female US blood donors, Jamaican blood donors and Jamaican STD patients.

data, particularly in older age groups, would be needed to examine this possibility further.

The data also showed that women were more likely to be seropositive than men (all p values < 0.01) and that this gender difference was apparent in each of the 3 study groups in essentially all age strata. In multivariate analysis, females were almost twice as likely as males to be HPV 16-seropositive, following adjustment for age and study group (OR = 1.9, 95% CI 1.5–2.4).

Risk factors for HPV 16 antibodies in STD patients

To further assess the relation of HPV 16 VLP antibodies with sexual behavior, we examined the association of self-reported sexual history with seroprevalence in the Jamaican STD patients. We were especially interested in determining whether within this population, universally at high risk of anogenital HPV infection and (as above) having high HPV 16 seroprevalence, ELISA results would further distinguish subjects with the highest levels of sexual experience.

Males were more sexually experienced than females. For example, 87% of males reported more than 5 partners as compared to 33% of females ($p < 0.001$) and males also had a greater average duration of sexual activity (14.2 years *vs.* 10.9 for females, $p < 0.001$). We observed, therefore, that the higher prevalence of antibodies in female (42%) compared with male (29%) STD patients existed despite the higher number of sex partners among men. In this connection, risk factors for HPV antibodies were studied separately by gender.

Table II shows the relationship between HPV 16 seropositivity and selected sexual characteristics. The main factor of interest was the lifetime number of sex partners because it was the factor most consistently associated with cervical HPV infection in DNA-hybridization studies (Bauer *et al.*, 1993; Hildesheim *et al.*, 1993; Wheeler *et al.*, 1993). In both men and women, seropositivity was significantly related to a higher lifetime number of sex partners, though the number of partners that conferred the increased risk differed by gender: for males, having 10 or more partners was associated with increased HPV 16 seroprevalence (34%) compared to males with fewer than 10 partners (21%, $p = 0.005$); HPV 16 seroprevalence rates were similar for female subjects reporting 1 or 2 to 4 lifetime partners but were significantly increased among females reporting 5 or more partners (46% compared to 36% in women with fewer than 5 partners, $p = 0.03$). In Table II, for convenience, the data are presented using a single category for males and females, showing a similar, albeit less statistically significant, trend in both groups. Interestingly, years of sexual activity in males ($p < 0.01$) and females ($p < 0.001$), but not age at first intercourse ($p > 0.6$), was also strongly associated with the frequency of HPV 16 serologic responses.

We conducted multivariate analyses to further examine these and other risk factors. As might be expected, age and years of sexual activity were highly correlated (Pearson's $r = 0.91$ for males and 0.97 for females) and, due to concerns regarding colinearity, the 2 factors could be studied only in separate multivariate models. In males, multivariate analyses showed that the relationship between greater lifetime number of sexual partners and HPV 16 seropositivity was independent of age ($p < 0.01$) and years of sexual activity ($p = 0.02$). Similarly, age and years of sexual activity were each associated with seroprevalence ($p < 0.05$ for both) independent of the number of sex partners.

Several additional factors involving sexual history during the past year were also significantly related to increased detection of HPV antibodies in men. This included the history in the past year of tears or bruises of the penis during sex ($p < 0.01$), a greater number of sexual partners ($p < 0.01$) and less regular use of condoms ($p < 0.05$). Notably, these 3 factors were somewhat interrelated. Specifically, having more sexual partners in the past year was associated with more frequent penile injury ($p = 0.008$) and greater condom use ($p = 0.001$), though condom use in the past year was not itself related to the frequency of penile injury ($p = 0.5$). In multivariate analyses, each of these parameters remained independently associated with HPV 16 seroprevalence and no significant interactions were detected when using appropriate interaction terms (all p values > 0.1).

Among female STD attendees, years of sexual activity was the variable most strongly associated with HPV 16 antibody positivity ($p = 0.001$). In multivariate analyses, years of sexual activity remained significantly associated with HPV 16 seropositivity ($p = 0.01$), whereas the relation of antibodies to greater lifetime number of sex partners lost statistical significance ($p = 0.27$). Unlike in men, no information regarding sexual behavior in the past year was obtained for female STD patients, and no additional behavioral factors were associated with detection of HPV antibodies. A history of prostitution showed a non-significant relation with increased HPV seroprevalence.

Almost all men and a random sample of about half the women studied were tested for infection with HIV and HTLV-I. No significant associations between infection with these retroviruses and antibody to HPV 16 VLPs was observed. However, retroviral infection rates were low and in women a possible relationship between HTLV-I and HPV seroprevalence of borderline statistical significance was non-significant after adjustment for age (or years of sexual activity) and lifetime number of sex partners ($p = 0.13$).

DISCUSSION

The failure of HPV DNA-hybridization studies to find high rates of cervical HPV infection among populations with high cervical cancer rates has long been noted to be inconsistent with the central role of HPV in cervical tumorigenesis (Kjaer and Jensen, 1992).

TABLE II – RISK FACTORS FOR HPV-16 VLP ANTIBODIES AMONG MALE AND FEMALE JAMAICAN STD SUBJECTS

	Males			Females		
	Number positive/ number tested	% positive	*p*	Number positive/ number tested	% positive	*p*
Age (years)						
<20	10/54	18.5		28/90	31.1	
20–24	29/112	25.9		38/117	32.5	
25–29	21/94	22.3		36/83	43.4	
30–34	25/72	34.7		34/71	47.9	
>34	27/72	37.5		34/66	51.5	
			<0.01			<0.001
Number of lifetime sex partners						
<5	12/50	24.0		98/274	35.8	
5–10	28/140	20.0		57/123	46.3	
11–20	32/94	34.0		5/10	50.0	
>20	37/110	33.6		0/1	0.0	
			0.02			0.07
Age at first intercourse (years)						
<14	47/175	26.9		26/58	44.8	
14–16	37/129	28.7		96/249	38.6	
>16	24/90	26.7		46/116	39.7	
			0.97			0.63
Years of sexual activity						
<5	7/38	18.4		26/102	25.5	
5–9	19/89	21.4		51/117	43.6	
10–14	21/97	21.7		29/77	37.7	
15–19	31/85	36.5		25/61	41.0	
>19	30/85	35.3		37/65	56.9	
			<0.01			<0.001
Number of sex partners in past year						
0–1	16/70	22.9				
2–5	63/258	24.4		n/a		
>5	30/68	44.1				
			<0.01			
Tear or bruise of penis during sex in past year						
Never	40/177	22.6				
Once	21/92	22.8		n/a		
Occasionally	37/105	35.2				
Half the time or more	11/22	50.0				
			<0.01			
Condom use during past year						
Never	29/92	31.5				
<Half the time	50/157	31.9		n/a		
Half the time or more	29/145	20.0				
			<0.05			
Ever used condoms						
No	14/59	23.7		48/115	42.0	
Yes	94/333	28.2		120/307	38.0	
			0.48			0.62
New sex partner in past month						
No	62/218	28.4		136/349	39.0	
Yes	46/176	26.1		33/77	42.9	
			0.61			0.53
History of previous STD						
No	34/121	28.1		97/257	37.7	
Yes	75/274	27.4		68/162	42.0	
			0.88			0.39
Number of previous STDs						
0	32/116	27.6				
1–2	47/191	24.6		n/a		
>2	29/78	37.2				
			0.22			
HIV-positive						
No	93/346	26.9		114/273	41.8	
Yes	8/21	38.1		6/14	42.9	
			0.26			0.94
HTLV-I-positive						
No	97/354	27.4		114/275	40.6	
Yes	3/10	30.0		8/13	70.0	
			1.00[1]			0.15
Ever accepted money for sex						
No	104/378	27.5		157/403	39.0	
Yes	5/18	27.8		11/21	52.4	
			1.00[1]			0.22
Ever paid for sex						
No	94/343	27.4				
Yes	15/53	28.3		n/a		
			0.89			
Anogenital warts						
No	98/356	27.5				
Yes	4/19	21.1		n/a		
			0.54[1]			

[1]Fisher's exact test. All other categorical comparisons are by Mantel extension test for trend or by Pearson's χ^2 test.

The favored explanation has been that HPV infection is transient, as is the detection of HPV DNA in anogenital tissues, making DNA hybridization a poor marker of population-based rates of exposure. In contrast, antibody responses to infection are likely to persist for a more extended period, making serology a potentially better measure of cumulative exposure to HPV and, possibly, a useful epidemiologic tool for comparing populations. Therefore, we compared the prevalence of antibodies to HPV 16, the most common and important oncogenic HPV type, among volunteer blood donors from Jamaica, a country with high cervical cancer rates, to the prevalence among donors in the United States, a country with relatively low cervical cancer rates. In addition, to demonstrate the strong sexual association of the antibody responses being measured, we contrasted these results to seroprevalence rates in Jamaican STD patients and, in these patients, examined risk factors for positive antibody responses.

We found that HPV 16 antibodies were significantly more common in Jamaican than in US blood donors. Specifically, after adjusting for age and gender, Jamaicans were approximately 4-fold more likely to be seropositive than their American counterparts. These data suggest that infection with HPV 16 is more common in men and women living in Jamaica than in the United States. Although the proportion of increased cervical cancer incidence in Jamaica attributable to higher rates of HPV infection cannot be determined from these data, it is likely that this as well as limited access to cervical cancer screening and treatment services are important contributing factors. US blood donors were more recently enrolled. However, increases in rates of HPV-related carcinomas of the anus and vulva in American women (cancers not prevented by contemporary Pap smear screening programs) (Reis *et al.*, 1997) strongly indicate that anogenital HPV infections have not decreased in recent years, making a period effect unlikely.

Our findings are consistent with 2 previous studies, both of which suggested that the prevalence of antibodies to HPV 16 VLPs is greater in individuals living in countries with high rates of

cervical cancer. Specifically, Nonnenmacher *et al.* (1995) showed that Colombian women enrolled as controls in a cervical cancer case-control study had higher HPV 16 seroprevalence than similar women enrolled in Spain, and Svare *et al.* (1997) demonstrated higher seroprevalence in male and female STD patients enrolled in Greenland than in Denmark. The current results, therefore, support these previous observations and extend that work by demonstrating that the relation of HPV 16 antibody prevalence with regional cervical cancer rates also applies to men and women commonly thought to be representative of the general population (*i.e.,* volunteer blood donors) (Strickler *et al.,* 1995).

Consistent with the strong relation of HPV infection with sexual history, HPV 16 VLP seroprevalence was far higher in male and female Jamaican STD patients than in either Jamaican or US blood donors. Moreover, in this group of men and women at high risk of sexually transmitted infection, we found that prevalence was further increased with greater number of sex partners and years of sexual experience. These data are particularly relevant because earlier DNA-hybridization studies of risk factors for HPV infection suggested that the lifetime number of sex partners was the parameter most consistently associated with cervical HPV infection (Bauer *et al.,* 1993; Hildesheim *et al.,* 1993; Wheeler *et al.,* 1993). The clear sexual association of HPV 16 seroprevalence does not exclude possible cross-reactivity with other HPV types. However, the findings indicate that the antibodies measured reflect sexually transmitted (anogenital) HPV.

Among men, sexual behavior in the past year was also significantly associated with HPV 16 seroprevalence. Men with a greater number of sexual partners in the past year and lower frequency of condom use during that time were more likely to be HPV 16-seropositive. This is of particular interest because studies of HPV infection and condom use, based on detection of HPV DNA, have yielded mixed results. Similar information regarding sexual history in the last year was not asked in the women's questionnaire, but having ever used a condom in one's lifetime was not associated with HPV seroprevalence in either men or women. Therefore, although the data strongly support the sexual relation of HPV 16 antibody detection, further study is necessary to confirm the protective effect of condom use. The findings suggest that serology may be a useful way to examine this issue in the future.

In contrast to the above, very recent sexual behavior, as reflected by report of a new sexual partner in the last month, was not associated with detection of antibodies in either men or women. This observation is consistent with results suggesting that antibodies are most strongly associated with persistent (as compared to recent) infections and that seroconversion may be delayed (Andersson-Ellstrom *et al.,* 1995; De Gruijl *et al.*, 1997). Several other sexual parameters were also not strongly associated with HPV antibody responses, including history of prostitution (a non-significant positive association in women and none in men) and the number of past STDs (a non-significant positive association in men, with no similar question asked of women). We also observed no relation between antibodies to HPV 16 and infection with HIV. This is consistent with a recent report in gay men, indicating that in groups at high risk for anogenital HPV, HIV infection did not seem to affect HPV 16 VLP seroprevalence (Hagansee *et al.*, 1997). Neither was there a clear relation with HTLV-I infection.

Another important observation was the association of HPV 16 VLP antibody prevalence with age. Among male and female STD patients, this relationship was an essentially monotonic, statistically significant increase with year of age, consistent with the idea that HPV serology is a measure of cumulative exposure. However, among the Jamaican and US blood donors, age-specific HPV antibody prevalence rates seemed to suggest a plateau after an initial increase. One possible interpretation is that among individuals who do not have continued frequent sexual contact with new partners lifetime exposure to HPV does not continue to increase substantially. Alternatively, antibodies may begin to wane over time in at least a subset of individuals. In the latter case, the continued increase of HPV antibodies with age in STD patients (but not blood donors) would reflect a combination of new infections and the effects of repeated sexual exposure, preventing HPV 16 antibody responses from beginning to wane. Much more data regarding the age-specific prevalence of HPV antibodies in different populations, especially older individuals, and cohort studies are needed to assess these issues more completely.

The lower prevalence of HPV-16 antibodies in men as compared to women was also notable in all groups tested. This included STD patients despite the fact that men had much greater sexual experience with significantly more sexual partners and longer duration of sexual activity. Confounding by other unmeasured sexual or behavioral factors may have contributed to the difference in these findings. However, it may also reflect an important underlying biologic difference between HPV infection in men and women. Specifically, anogenital HPV infection in males may more likely involve squamous tissues with limited immunosurveillance (*e.g.,* the external surfaces of the penis) relative to the mucosal epithelial tissues commonly infected in women (*e.g.,* the cervical transformation zone). In this connection, men who reported frequent tears or bruises of the penis during sex in the past year had significantly higher HPV 16 VLP seroprevalence than other men. Tears in the skin of the penis might result in higher seroprevalence by causing bleeding and increased interaction between the immune system and HPV-infected cells. Alternatively, penile tears might be a marker of sexual history (*e.g.,* occurring more frequently in the presence of ulcerative STDs) or result in increased HPV infections by exposing the basal epithelium, the target cells of anogenital HPV. Probably each of these possibilities plays some role.

In summary, our current findings suggest that HPV 16 VLP serology is a useful biomarker for measuring cumulative lifetime exposure to sexually transmitted anogenital HPV infections and may be particularly applicable to population-based studies. In this connection, we detected much higher exposure to HPV 16 in Jamaicans than in Americans, consistent with Jamaica's higher cervical cancer incidence rates. Jamaican STD patients had especially high HPV 16 VLP seroprevalence, and the data in these patients showed that serology may be useful as a marker of sexual anogenital HPV exposure in women and in men (a group difficult to test by DNA-hybridization methods). The prevalence of HPV antibodies was lower in males than females, however, suggesting that there may be diminished immune surveillance of the epithelial tissues commonly infected by HPV in men. Whether this might affect the usefulness of HPV vaccines in men requires further examination.

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